PATENT
ATTORNEY DOCKET NO. 07043/015007

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : M. Allen Northrup, *et al.* Art Unit: Not yet assigned
Serial No.: Not yet assigned Examiner: Not yet assigned
Filed : Herewith
Title : **MICROFABRICATED REACTOR**

Assistant Commissioner for Patents
Washington, DC 20231

PRELIMINARY AMENDMENT

Prior to examination of the application filed herewith, please amend the application as follows:

In the specification:

At page 1, just prior to the heading "Background of the Invention" which starts on line 8, please insert the following heading and subsequent paragraph:

-- Statement of Rights to Inventions Made Under Federally-Sponsored Research and Development

This invention was made with Government support under Grant No. ECD-86-14900 (BSAC) awarded by the National Science Foundation. The Government has certain rights to this invention.--

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Chris Hamer
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Page 1, lines 3-4, replace "filed January 18, 1990," with --issued July 14, 1992--.

Page 3, line 31, insert --,-- after "reactions".

Page 3, line 31, replace "(PCR)," with --(PCR) or the ligase chain reaction,--.

Page 8, line 20, after "samples." insert --Samples may be less than a milliliter, less than a microliter, or less than a picoliter.--.

Page 13, line 25, replace "which" with --with--.

Page 14, line 1, replace "pre-determined" with --predetermined--.

Page 14, line 7, replace "need" with --needle--.

Page 14, line 13, replace "FIG. 2" with --FIG. 1--.

Page 15, line 26, replace "channel 34" with --channel 32--.

Page 18, line 43, replace "50bl and 50br" with --50b--.

Page 19, lines 2, 5, 20, 23, and 34, replace "nitride" with --silicon-nitride--.

Page 19, lines 24-28, replace "Left and right top access holes 64tl, 64tr, 65tl, and 65tr provide access to the polycrystalline layer 54t. Similarly, left and right bottom access holes 64bl, 64br, 65bl, and 65br provide access to the bottom polycrystalline layer 54b." with

--Left and right bottom access holes 64bl, 64br, 65bl, and 65br provide access to the bottom polycrystalline layer 54b (and similarly for the top

access holes 64tl, 64tr, 65tl, and

65tr, (not shown)).--.

Page 20, lines 2-3, replace "50bl, 50br, 50tl and 50tr" with --50b and 50t--.

Page 20, line 18, replace "65tr," with --65tr (labelled in FIG. 4(d) but not in FIG. 4(e)),--.

Page 21, line 16, replace "60tl and 60tr" with --60bl and 60br--.

Page 24, line 3, replace "64tly" with --64bly-- and "top" with --bottom--.

Page 24, line 4, replace "66t" with --66b-- and "60tly" with --60bl--.

Page 24, line 5, replace "62t" with --62b--.

Page 24, line 7, replace "70t" with --70b--.

Page 24, line 9, replace "64tlx" with --64blx--.

Page 27, line 24, insert --such as a protein-- after "organic,".

Page 27, line 25, insert --the reactants may be any type of large molecules, proteins, polymers or biochemical compounds;-- after "inorganic;".

Page 27, line 27, insert --the number of thermal cycles may be greater than ten, or greater than twenty five;-- after "means;".

<u>In the Claims:</u>

Claim 1(Amended) An instrument for controlling [a chemical reaction of reagents comprising: integrated

microfabricated elements, said elements including a first reagent chamber for containment of a first one of said reagents, and a means for manipulation of a parameter of said reaction] at least one chemical reaction, comprising:

a) an array of chambers for containment of the reaction including:

at least one chamber for preparing a sample for use in said reactions; at least one chamber for adding or removing reagents involved in said reactions;

at least one channel interconnecting said chambers;

b) a temperature controller of said reaction; and

c) a product analysis chamber coupled to and adapted to perform analysis of said reaction.

Please cancel claims 2-92.

Please add the following new claims:

--93. The instrument of claim 1, wherein said means for analysis is selected from the group consisting of: sequencing of target species, DNA fingerprinting, physical mapping of target species, DNA library analysis, electrochemical detection, and hybridization detection.

94. The instrument of claim 1, wherein said sample is selected from the group consisting of: intact cells, fixed cells, lysed

cells, microorganisms, and tissue.

95. The instrument of claim 94, wherein sample preparation yields a specific nucleic acid target molecule.

96. The instrument of claim 1, wherein said sample preparation includes sorting specific cell types.

97. The instrument of claim 1, wherein said chambers are constructed on a single substrate.

98. The instrument of claim 1, wherein said reaction is controlled at a constant temperature.

99. The instrument of claim 98, wherein said reaction is in vitro transcription.

100. The instrument of claim 1, wherein said reaction is controlled by thermal cycling.

101. The instrument of claim 100, wherein said reaction is a chain reaction.

102. The instrument of claim 101, wherein said reaction is a polymerase chain reaction.

103. The instrument of claim 101, wherein said reaction is a

ligase chain reaction.

104. An instrument for controlling at least one chemical reaction, comprising:

a) an array of chambers for containment of the reaction including:

at least one chamber for preparing a sample for use in said reactions;

at least one chamber for adding or removing reagents involved in said reactions;

at least one channel interconnecting said chambers;

a transferring mechanism coupled to said chambers by way of said channel;

b) a temperature controller coupled to said instrument;

c) at least one chamber for analysis of products of said at least one chemical reaction.

105. The instrument of claim 104, wherein said means for analysis is selected from the group consisting of: sequencing of target species, DNA fingerprinting, physical mapping of target species, DNA library analysis, electrochemical detection, and hybridization detection.

106. The instrument of claim 104 in which said means for analysis utilizes a predetermined array of oligonucleotides.

107. The instrument of claim 106 in which said array in used in hybridization techniques.

108. The instrument of claim 104, wherein said means for analysis includes purification of said reaction products.

109. The instrument of claim 108, wherein said purification is performed by electrophoresis.

110. The instrument of claim 108, wherein said purification is performed by chromatography.

111. The instrument of claim 104, wherein said sample is selected from the group consisting of: intact cells, fixed cells, microorganisms, and tissue.

112. The instrument of claim 111, wherein sample preparation yields a specific nucleic acid target molecule.

113. The instrument of claim 104, wherein said sample preparation includes sorting specific cell types.

114. The instrument of claim 104, wherein said chambers are constructed on a single substrate.

115. The instrument of claim 104, wherein said reaction is controlled at a constant temperature.

116. The instrument of claim 115, wherein said reaction is in vitro transcription.

117. The instrument of claim 104, wherein said reaction is controlled by thermal cycling.

118. The instrument of claim 117, wherein said reaction is a chain reaction.

119. The instrument of claim 118, wherein said reaction is a polymerase chain reaction.

120. The instrument of claim 118, wherein said reaction is a ligase chain reaction.

121. The instrument of any of claims 113-120, wherein the reagents include labeled primers for subsequent identification of reaction products.--

REMARKS

Claim 1 has been amended to more fully claim the invention. Claims 2-92 have been cancelled. Claims 93-121 have been added. These claims find full support in the original specification at, for example, page 1, line 9-20; page 3, lines 5-34; page 4, lines 1-30; page 5, lines 1-27; page 9, lines 24-35; page 10, lines 1-36; the entirety of pages 11-13; and page 14, lines 1-4. These claims are presented to more fully claim

what applicant regards as the invention.

Respectfully submitted,

Date: July 24, 1997

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MICROFABRICATED REACTOR

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RELATED APPLICATIONS

This application is related to U.S. Patent 5,129,261, Serial No. 07/467,412, filed January 18, 1990 and application Serial No. 07/162,193, filed February 29, 1988, now abandoned, for a Plate-mode Ultrasonic Sensor. The entire disclosures of these applications are hereby incorporated by reference.

BACKGROUND OF THE INVENTION

The present invention relates generally to instruments for chemical reaction control, product and reactant manipulations, detection of participating reactants and resultant products, and more particularly to integrated microfabricated instruments which perform microscale chemical reactions involving precise control of parameters of the reactions. The parameters of the reaction controlled by the instrument may be temperature, pressure, concentration of reactants, the intensity or frequency of incident light, electromagnetic fields, or ultrasonic pressure waves, etc.

The term "integrated microfabrication" is used herein to refer to all processes used for batch production of semiconductor microelectronics, and all related microfabrication processes such as LIGA (see R. S. Muller, R. T. Howe, S. D. Senturia, R. L. Smith, and R. M. White, ed. MICROSENSORS, IEEE Press, 472 pages, 1990). Microfabrication technologies include, but are not limited to, sputtering, electrodeposition, low-pressure vapor deposition, photolithography and etching. Microfabricated devices are usually formed on crystalline semiconductor substrates such as silicon or gallium arsenide. Noncrystalline materials such as glass or certain polymers may be used although crystalline materials provide certain advantages. The shapes of crystalline devices can be precisely controlled since etched surfaces are generally crystal planes, and crystalline materials may be bonded by processes such as fusion at elevated temperatures or the field-assisted method (Mallory bonding). Materials which are not semiconductors, such as quartz or glass, may be used, though semiconductor materials provide the advantage that electronic circuitry may be integrated into the system by the use of conventional integrated-circuit fabrication techniques.

Monolithic microfabrication technology now allows the production of electrical, mechanical, electromechanical, optical, chemical and thermal devices including pumps, valves, heaters, mixers and species detectors for microliter to nanoliter quantities of solids, liquids and gases. Microscale sensors include optical waveguide probes and ultrasonic flexural-wave sensors. The integration of these devices into a single system allows for the batch production of microscale reactor-based analytical instruments. Integrated microinstruments

may be applied to biochemical, inorganic, or organic chemical reactions to perform biomedical and environmental diagnostics, and biotechnological processing and detection.

Such integrated microfabricated devices can be manufactured in batch quantities with high precision, yet low cost, thereby making recyclable and/or disposable single-use devices practical. Alternatively, the instrument may consist of an array of reaction instruments which are to operate in parallel to simultaneously perform a number of related reactions. Operation of such instruments is easily automated, further reducing costs. Since the analysis can be performed *in situ*, the likelihood of contamination is very low. Because of the inherently small sizes of such devices, the heating and cooling can be extremely rapid, and the devices can have very low power requirements. Such devices may be powered by batteries or by electromagnetic, capacitive, inductive or optical coupling.

Small volumes and high surface-area to volume ratios provide microfabricated reaction instruments with a high level of control of the parameters of a reaction. Heaters may produce temperature cycling or ramping, sonochemical and sonophysical changes in conformational structures may be produced by ultrasound transducers, and polymerizations may be generated by incident optical radiation.

Synthesis reactions, and especially synthesis chain reactions such as the polymerase chain reaction (PCR), are particularly well-suited for microfabricated reaction instruments. PCR can selectively amplify a single molecule of DNA (or RNA)

of an organism by a factor of 10^6 to 10^9. This well-established procedure requires the repetition of heating (denaturing) and cooling (annealing) cycles in the presence of an original DNA target molecule, specific DNA primers, deoxynucleotide triphosphates, and DNA polymerase enzymes and cofactors. Each cycle produces a doubling of the target DNA sequence, leading to an exponential accumulation of the target sequence. PCR-based technology has been applied to a variety of analyses, including environmental and industrial contaminant identification, medical and forensic diagnostics, and biological research.

The procedure involves: (1) processing of the sample to release target DNA molecules into a crude extract; (2) addition of an aqueous solution containing enzymes, buffers, deoxyribonucleotide triphosphates (dNTPS), and oligonucleotide primers; (3) thermal cycling of the reaction mixture between two or three temperatures (e.g., 90-96, 72, and 37-55°C); and (4) detection of amplified DNA. Intermediate steps which incorporate signal-producing and/or surface-binding primers, or which purify the reaction products, via, for example, electrophoresis or chromatography may be introduced. A problem with standard PCR laboratory techniques is that the PCR reactions may be contaminated or inhibited by introduction of a single contaminant molecule of extraneous DNA, such as those from previous experiments, or other contaminants, during transfers of reagents from one vessel to another.

PCR reaction volumes are presently typically on the order of 50 microliters. A thermal cycle typically consists of heating a sample to a first temperature, maintaining the sample at the first temperature, cooling the sample to a second

lower temperature, and maintaining the temperature at that lower temperature. The rate at which the sample is heated is generally limited by the heater rather than the rate of heat transfer to the sample. Presently, each of the four stages of a thermal cycle requires approximately one minute, and the time required for twenty to forty complete thermal cycles is therefore from about one to three hours. The cycling time has been reduced by performing the PCR reaction in capillary tubes (see C. T. Wittwer, G. C. Fillmore, and D. J. Garling, Analytical Biochemistry, **186**, pp. 328-331 (1990)). A high-power forced air heater was used to heat the tubes. The thinnest capillary tubes contained a sample volume of about ten microliters. Each cycle consisted of a heating step, a waiting period, a cooling step and another waiting period, and each step required approximately fifteen seconds.

Although the PCR reaction requires thermal cycling of the reagents, any reaction that benefits from precise temperature control, and/or rapid thermal cycling, thermal ramping, or any other temperature variation of reagents with time (hereinafter to be referred to as temperature programming) will be well suited for the microfabricated reaction instrument of the present invention.

An object of the present invention is therefore to provide a integrated microfabricated reactor.

Another object of the present invention is to provide a reactor-based instrument for inorganic, organic and biochemical reactions, and in particular for diagnostics.

Another object of the present invention is to provide a reactor which provides high-precision control of reaction parameters.

Another object of the present invention is to provide a reactor which provides high-precision temperature control.

Another object of the present invention is to provide a reactor which provides rapid high-precision thermal cycling, ramping or programming.

Another object of the present invention is to provide a closed system reactor which is self-contained, e.g. which is shipped from the factory containing the reagents, thereby minimizing the susceptibility to contamination.

Another object of the present invention is to provide low-cost reaction and/or detection systems.

Another object of the present invention is to provide an instrument for *in situ* reactions which may be powered by incident electromagnetic radiation or batteries.

Another object of the present invention is to provide arrays of microfabricated reaction chambers which may operate in parallel or series.

Additional objects and advantages of the invention will be set forth in the description which follows, and in part will be obvious from the description, or may be learned by practice of the invention. The objects and advantages of the invention may be realized and obtained by means of

the instrumentalities and combinations particularly pointed out in the claims.

SUMMARY OF THE INVENTION

The present invention is directed to an instrument for *in situ* chemical reactions in a microfabricated environment. The instrument is especially advantageous for biochemical reactions which require high-precision thermal cycling, particularly DNA-based manipulations such as PCR, since the small dimensions typical of microinstrumentation promote rapid cycling times.

The present invention provides a reaction instrument comprised of integrated microfabricated elements including a reagent chamber and a means for manipulating the reaction of the reagents.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings, which are incorporated in and constitute a part of the specification, schematically illustrate a preferred embodiment of the invention and, together with the general description of the preferred embodiment given below, serve to explain the principles of the invention.

Figure 1 shows a cut-away perspective view of a reaction instrument of the present invention mounted in a power source/control apparatus.

Figure 2 is a schematic of a reaction instrument of the present invention.

Figure 3 shows a cross-sectional view of a reaction chamber of the present invention.

Figures 4(a) through 4(f) show cross-sectional views of the stages of fabrication of a reaction chamber of the present invention.

Figure 5 shows a top view of a reaction chamber (dashed outline) below the piezoelectric and ground plane layers.

Figure 6a shows the typical flow velocity profile field for a fluid forced through a conduit with static bottom and side surfaces, and Figure 6b shows the flow velocity profile for a fluid pumped through a conduit by a flexural-wave pump.

Figure 7 shows gel electrophoretic results verifying the amplification of an HIV genetic sequence in a microfabricated reaction chamber of the present invention.

DESCRIPTION OF THE PREFERRED EMBODIMENT

The microinstrument of the present invention has integrated microfabricated components that perform reactant and product manipulations and detection on microliter to picoliter samples. By the selection and integration of appropriate microfabricated devices, a precise and reliable reaction and analysis instrument for PCR-based diagnostics is implemented.

The instrument may be fabricated in a wide variety of different forms. Many microinstruments may be manufactured on a single wafer and can run in parallel, allowing the processing and analysis of several target agents and controls simultaneously. Individual small and disposable dies, each a complete microinstrument, may be fabricated. The device may be fabricated to allow a continual flow of reagents through the instrument. A reagent reservoir of the

microinstrument may have a thin silicone rubber wall so that the reagent may be inserted into the microinstrument by a hypodermic needle. Alternatively, a needle may be integrated into the microinstrument so that a patient can be pricked by the needle and a blood sample, or any other type of body fluid, will directly enter the instrument. An integrated needle can also be used to extract body fluids from plants and animals. The reagent may also be loaded into the microinstrument by pipetting, a modified ink-jet printing process, or other means at the factory. The reagent may be lyophilized or dried, or previously stored in the chamber.

Detection signals may be processed and stored by integrated microelectronic devices so that result interpretation and control mechanisms (which may utilize feedback) can be integrally contained on the microinstrument. The low power needs of microinstrumentation allows such systems to be powered by incident electromagnetic radiation, low voltage batteries, or incident optical radiation converted to electrical energy by on-board photocells.

The components of a microinstrument may include reservoirs for retaining reagents, agitators and mixers, heaters to perform denaturing and annealing cycles, pumps, optical and/or electromechanical sensors to discriminate reagents, and reagent separators. Microheaters may be resistive heaters consisting of materials such as polysilicon patterned onto and made an integral part of the microstructure. The micropumps may be Lamb-wave devices (see U.S. Patent No. 5,006,749, R.M. White, 1991), electrokinetic pumps, or other microfabricated pump structures. The microdetection

instruments may be fluorescence-based optical fiber spectroscopes which utilize microfabricated light sources and detectors (e.g., LEDs or diode lasers and detectors); Lamb-wave sensors (see U.S. patent 5,129,261, Serial No. 07/467,412 filed January 18, 1990, and application Serial No. 07/775,631 filed October 10, 1991); electrochemical detection of biochemical molecules by surface plasmon resonance or other processes involving immobilized biochemicals; electrochemical sensing devices; or other appropriate detection methodologies. Surface treatments may be applied to components of the device for reaction enhancement, product separation, and species detection. The surface treatments can be based on numerous well-known procedures such as silanol-based derivatizations or other appropriate treatments. Chemical species separators can utilize microelectrophoresis either in a capillary or within a gel, or can be based on other appropriate methodologies.

One embodiment of the present invention performs the polymerase chain reaction (PCR). The minute reagent volumes and the specific reaction sequence of the PCR technique play favorably into the advantages of the present invention. The integrated microsystem provides a highly automated, miniaturized, analytical instrument for very rapid *in situ* analyses and production of a variety of samples. Integrated microfabricated PCR instruments are capable of performing, *in situ*, many reactions and manipulations with precise control of temperature, evaporation, small-volume reagent delivery, product separation, isolation and detection. Such highly automated technologies should greatly expedite the use of DNA-based technologies for biomedical (e.g., the Human Genome Project); environmental (e.g.,

contaminant identification); industrial (e.g., biotechnology); and forensic applications. The principles applied and problems solved in developing a PCR instrument may of course be applied to other chemical reactions, analyses, and syntheses, especially other biochemical reactions, analyses, and syntheses.

PCR in a microdevice can be just one step in a series of manipulations leading to the diagnostic detection of a variety of target species or the use of PCR products in genetic engineering. Physical and chemical treatments such as those described below can also be incorporated into the pre-PCR and post-PCR phases of microdevice-based treatments to augment the reactions *in situ*. Amplification via PCR yields products that may be subject to further enhancement or utilized for detection of other chemicals. Physical and chemical control via microdevices of biological cells and reagents prior to and after the production of PCR products will expand the number of the potential applications of DNA-based processes and analyses.

Pre-PCR manipulation of target cells or microorganisms can be accomplished with microdevices of the present invention. For example ultrasonic waves may be applied to disrupt and expose cell components through lysis, and to unravel large or long chain molecules such as DNA and proteins via disruption of secondary structure. Cell lysis may also be induced electrically or chemically. Ultrasonic waves and surface chemistry treatments can be used to manipulate cells and cell-contents, as can chemical treatment by stirring and/or mixing reagents from other chambers on the microinstrument. Sonication on a macro-scale in conjunction with

agitating microparticles, for example, has been used to facilitate the extraction of DNA from parafin-embedded fixed cells (M. J. Heller, L. J. Burgart, C. J. Ten Eyck, M. E. Anderson, T. C. Greiner, and R. A. Robinson, Biotechniques, **11**, #3, 1991, pp. 372-377). Strategies similar to this which rely on the inherent properties of a microdevice can be used to process intact cells, microorganisms, tissues, and other analytical samples for PCR and subsequent techniques.

Potential post-PCR treatments by the microdevice are also numerous. It should be noted that PCR is often an integral part of the potential application of a device to further biotechnological manipulations and analyses. Once PCR has been performed, post-PCR manipulations can lead to a myriad of possible microdevice-based DNA analyses and treatments. A few examples of such analyses are: large-scale and small-scale DNA sequencing of target species, cell-typing, analysis of PCR products with DNA probes, DNA recombination, DNA fingerprinting, DNA cloning, cell cloning, physical mapping of genes, incorporation of genetic vectors, genetic therapy, treatment and testing of biotechnological processes, and the maintenance of DNA libraries. Such analyses can lead to the use of DNA as vectors to produce cells or other biological entities to make desired products such as proteins or other compounds, or it can be used to produce DNA for use in therapies or biotechnological processes.

PCR products may also be manipulated in order to be incorporated into genetic engineering vectors such as plasmids. The vectors may subsequently be incorporated into target cells for the production of desired compounds. The target cells or moieties and reagents can be stored in

reservoirs on the device and released for exposure to the vectors when the proper physical/chemical conditions have been established. One other potential application would be the *in situ (in vitro* or *in vivo*) release of PCR products for direct genetic therapy or manipulations. Direct DNA sequencing of PCR products (single or double-stranded) can be accomplished with the use of unique temperature, enzymatic, or other separation schemes and detection methodologies; all of which can be incorporated into a microdevice.

Detection windows, reflective and absorptive surfaces, optic sources and other optical components can be fabricated and integrated onto a microdevice instrument, providing optical detection capabilities. The status of a reaction may be monitored by illuminating the reagents through an optical window and measuring absorption and/or luminescence. A waveguide may be fabricated by depositing a plane of transparent material between semiconducting planes, or by bonding two wafers together, at least one of the wafers having a transparent surface layer. The optical path of the waveguide is parallel to the fabrication surface. Alternatively, a window which an optical path parallel to the normal vector of the fabrication surface may be constructed using standard patterning techniques. Data analyses can be accomplished with on-board electronics which may provide electronic or optical output.

Analysis of PCR products, sequences of target DNA, or synthetic analogues in microdevices can be accomplished with the manipulative capabilities of microfabricated electrical and mechanical machines. For example, two-dimensional

arrays of pre-determined DNA sequences (probes) can be used to detect or verify PCR products, and their subsequent analyses can be accomplished with microdevices.

As shown in FIG. 1, an embodiment 20 of the present invention is shown above a recess 105 in a power source/control system 100. A hypodermic need 110 is shown inserting a sample through a silicone rubber window 120 into a reaction instrument 20. The reaction is controlled and powered by: inductive coupling, such as that between coil L_{CL} in the instrument 20 and a magnetic coil 130 as shown in FIG. 2; by capacitive coupling, such as that between the plates of capacitor C_3 and plates 140a and 140b; and by electromagnetic coupling between resonant circuit (not shown) in the instrument 20 and a radio-frequency antenna 135.

A schematic of a preferred embodiment 20 of the present invention is shown in FIG. 2. Three reagent chambers 10, 12 and 14 contain reactants. One chamber 10 contains the DNA primers, one chamber 12 contains the polymerase, and one chamber 14 contains the nucleotides and any detection-tag molecules, such as magnetic beads. The contents of the chambers 10, 12 and 14 have been loaded at the factory. The target DNA molecule is placed in reagent chamber 10 by insertion of a hypodermic needle or the like through silicone rubber window 120. The window 120 may alternatively be composed of any other type of appropriate natural or synthetic elastomer. The reactants in the reagent chambers 10, 12 and 14 are connected by channels 22, 24 and 26 to a reaction chamber 30. Typically the chambers 10, 12, 14 and 30 have a volume ranging from microliters to nanoliters. The channels 22, 24 and 26 are

equipped with Lamb-wave pumps LW_1, LW_2 and LW_3, respectively, for pumping the reactants in the reactant chambers 10, 12 and 14 in the directions of the arrows to the reaction chamber 30. The Lamb-wave pumps may be located on any wall, or on multiple walls, of the channels 22, 24 and 26. Lamb-wave pump LW_1 is connected to a capacitor C_1. Similarly the other two Lamb-wave pumps LW_2 and LW_3 are connected to capacitors C_2 and C_3, respectively.

The surface tension across the narrow midsections of the channels 22, 24 and 26 prevents the reactants from flowing into the reaction chamber 30 until pumping is initiated. The surfaces of the channels 22, 24 and 26 may be treated to raise the surface tension thereby further inhibiting flow of reagents when the pumps LW_1, LW_2 and LW_3 are not activated.

The reaction chamber 30 may be equipped with a Lamb-wave transducer LW_C and a heater H_C. The Lamb-wave transducer is connected to inductor L_{CL}. The heater H_C is connected to a resonant circuit consisting of an inductor L_{CH} and a capacitor C_{CH}. The Lamb-wave transducer LW_C acts as an agitator, mixer, or sonochemical inducer.

A channel 32 connects the reaction chamber 30 to a detection chamber 34. The channel 34 is equipped with a Lamb-wave pump LW_{DP}, which is connected to a resonant circuit consisting of an inductor L_{DP} and a capacitor C_{DP}. The detection chamber 34 is equipped with a Lamb-wave sensor LW_D. The Lamb-wave sensor LW_D is connected to a capacitor C_D.

For ease of notation, an exemplary Lamb-wave device chosen from the set LW_1, LW_2, LW_3, LW_C, LW_{DP}, and LW_D will be denoted by LW and the corresponding capacitor and/or inductor electrically connected to the Lamb-wave device will be denoted by C and L, respectively, hereinafter. Lamb-wave transducers have high mechanical Q values and can therefore be powered by only a narrow range of alternating voltage frequencies. The Lamb-wave pumps LW_1, LW_2, and LW_3, and the Lamb-wave sensor LW_D are powered capacitively by generating an electric field between the plates 140a and 140b at the resonant frequencies of the Lamb-wave transducers LW_1, LW_2, LW_3, and LW_D. The alternating frequency electric fields generate alternating frequency voltages across the capacitors C_1, C_2, C_3 and C_D, and Lamb waves at this frequency in the transducers LW_1, LW_2, LW_3 and LW_D. But because the transducers LW_1, LW_2, LW_3, and LW_D have high Q values, only when the frequency of the imposed field is near the resonant frequency of a transducer does the transducer vibrate with any substantial magnitude. Similarly, the Lamb-wave mixing chamber transducer LW_C is powered by an alternating frequency magnetic field generated by the coil 130 at the mechanical resonant frequency of the transducer LW_C. The heater H_C and the Lamb-wave pump LW_{DP} are activated by directing an electromagnetic wave from the antenna 135 to the resonant circuits C_{CH} and L_{CH}, and C_{DP} and L_{DP}, respectively. The frequency of the incident electromagnetic radiation must correspond to the resonant frequency of the electrical elements C_{DP}, L_{DP} and LW_{DP}, and must also correspond to the mechanical resonant frequency of the transducer LW_{DP}, to activate the pump LW_{DP}. The frequency of the incident electromagnetic radiation must correspond to the resonant frequency of the

electrical elements C_H, L_{CH} and H_C to activate the heater H_C.

The PCR reaction is initiated by pumping the reagents in the reagent chambers 10, 12 and 14 along the directions of the arrows to the reaction chamber 30 by activating the reagent pumps LW_1, LW_2 and LW_3. A series of approximately twenty to forty thermal cycles are then initiated, during each cycle the temperature of the reactants in the reaction chamber 30 goes from 55°C to 96°C, and back to 55°C, for example. The temperature of the reaction chamber 30 is determined by the power of the incident electromagnetic signal at the frequency corresponding to the resonant frequency of the circuit comprised of the capacitor C_{CH}, and the inductor L_{CH}, together with the heater H_C. The reaction chamber 30 Lamb-wave device LW_C acts as an agitator or mixer as described below to mix the reagents and promote the reaction.

When the thermal cycling is complete the contents of the reaction chamber 30 are pumped by Lamb-wave pump LW_{DP} in the direction of the arrow to the detection chamber 34. The preferred embodiment utilizes a Lamb-wave sensor LW_D. Alternatively, the detection chamber may be provided with an optical window and testing may be performed by fluorescence-based or absorption-based optical spectroscopy.

A cross-sectional view taken along line 3-3 of FIG. 2 of the reaction chamber 30 is shown in FIG. 3. FIGS. 4(a)-(f) show cross-sectional views of the bottom half of the chamber during successive stages of formation of the chamber 30 from a silicon substrate 50b. A similar set of stages are involved in the fabrication of the top portion of the chamber

30. Once fabricated, the top and bottom portions may be bonded together by Mallory bonding.

The chamber cavity 31 is bounded by a ceiling 70t, a floor 70b, and side walls consisting of silicon sections 50t and 50b and silicon nitride sections 49tl, 49bl, 49tr and 49br. The height of the chamber cavity 31 is approximately 0.5 mm, and the width and length of the chamber are approximately 4 mm or less. The whole instrument 20 may fit on a wafer as small as 1 cm x 1 cm x 0.5 cm.

The indentations in the silicon substrates 50b and 50t are formed by coating one side of the substrates 50b and 50t with silicon nitride layers 52b and 52t, and patterning silicon nitride layers on the other sides of the substrates 50b and 50t to form sections 49bl, 49br, 49tl, and 49tr, respectively, as shown in FIGS. 3 and 4(a). The silicon-nitride layers 52t and 52b are preferably applied by low-pressure chemical-vapor deposition of the silicon-nitride, and are preferably a low-stress nitride. The thickness of silicon-nitride layers 52t and 52b is chosen to provide a balance between the mechanical strength of the layers which increases with thickness, and the sensitivity of the Lamb-wave detector which decreases with thickness. The thickness is also chosen to provide practical resonant frequencies for the device. The thickness of the silicon-nitride layers 52t and 52b is preferably about 3 μm, plus or minus ten percent.

The system is etched, for example with the wet chemical etchant KOH, to create a cavity in the silicon substrate 50 as shown in FIG. 4(b). The remaining sections of silicon 50bl and 50br form portions of the side walls of the cavity 31.

Located outside the reaction chamber cavity 31 to the exterior of nitride layers 52b and 52t are top and bottom polycrystalline silicon layers 54t and 54b, respectively. The polycrystalline layers 54t and 54b are deposited on the nitride layers 52b and 52t by chemical vapor deposition and selectively patterned. The bottom portion of the chamber 30 with the patterned polycrystalline layer 54b on the silicon nitride layer 52b is shown in FIG. 4(c). The thickness of the polycrystalline layers 54t and 54b is preferably between 2000 and 4000 angstroms, and more preferably 3000 angstroms, plus or minus five percent.

Top and bottom barrier layers, composed of an insulating material such as low-stress silicon nitride or silicon dioxide, are deposited by low-temperature oxidation and patterned. The lower barrier layers 58bl, 58bcl, 58bc, 58bcr and 58br lie to the exterior of the polycrystalline layer 54b and the nitride layer 52b as shown in FIG. 4(d). Similarly, the upper barrier layers 58tl, 58tcl, 58tc, 58tcr, and 58tr lie to the exterior of the polycrystalline layer 54t and the nitride layer 52t. Left and right top access holes 64tl, 64tr, 65tl, and 65tr provide access to the polycrystalline layer 54t. Similarly, left and right bottom access holes 64bl, 64br, 65bl, and 65br provide access to the bottom polycrystalline layer 54b. The thickness of the barrier layers 58tl, 58tcl, 58tc, 58tcr, and 58tr, (to be collectively referred to as 58t) and 58bl, 58bcl, 58bc, 58bcr and 58br (to be collectively referred to as 58b) is preferably between 1000 and 5000 angstroms, and more preferably 2500 angstroms, plus or minus ten percent. The nitride layers 52b and 52t and the barrier layers 58b and 58t thermally

isolate the polycrystalline layers 54b and 54t from the high conductivity silicon layers 50bl, 50br, 50tl and 50tr, thereby increasing the effectiveness of the heaters formed by the polycrystalline layers 54b and 54t.

As shown in FIGS. 3 and 4(e), top left and right conducting leads 56tl and 56tr, bottom left and right conducting leads 56bl and 56br, top left and right transducers 60tl and 60tr, bottom left and right transducers 60bl and 60br, top left and right four-contact resistance monitoring leads 67tl and 67tr, and bottom left and right four-contact resistance monitoring leads 67bl and 67br are then patterned onto the device. Through access holes 64tl and 64tr, the leads 56tl and 56tr make electrical contact with the polycrystalline layer 54t, and similarly for the bottom section of the chamber 30. Through access holes 65tl and 65tr, the leads 67tl and 67tr make electrical contact with the polycrystalline layer 54t, and similarly for the bottom section of the chamber 30. The top polycrystalline layer 54t is therefore electrically connected to leads 56tl, 56tr, 67tl and 67tr. Similarly, the bottom polycrystalline layer 54b is electrically connected to lead 56bl, 56br, 67bl and 67br. Current passing through polycrystalline layers 54t and 54b generates heat. Therefore, the temperature of the chamber 30 can be controlled by the amount of voltage applied across the top and bottom leads 56tl and 56tr, and 56bl and 56br, respectively. Because the exterior temperature is generally below that of the chemical reactions the system is cooled by the ambient air and cooling elements are not generally required.

The temperature of the system is monitored by measurement of the resistance of the polycrystalline layers 54t and 54b by connecting leads 67tl and 67tr, and 67bl and 67br of four-contact resistance measuring circuits (not shown) to the top and bottom polycrystalline layers 54t and 54b, respectively. The resistance increases linearly with temperature.

FIG. 5 depicts a view of the bottom of the chamber 30 subsequent to the deposition of the transducers 60bl and 60br, leads 56bl and 56br, and four-contact resistance leads 67bl and 67br, as shown in FIG. 4(e). For clarity not all transducer leads 64blx, 64bly, 64brx and 64bry shown in FIG. 5 are depicted in FIGS. 3, 4(e) and 4(f). Only six fingers per transducer 60tl and 60tr are shown in FIGS. 5 for clarity, though transducers having approximately twenty or more fingers are preferred. The cavity 31 and channels 24 and 32 lay beneath the barrier layer 58b in the region between the dashed lines 29 and the crossbars of the T-shaped leads 56bl and 56br. The access holes 64bl and 64br (not shown in FIG. 5) lie beneath the cross-bars of the leads 56bl and 56br, and the access holes 65bl and 65br (not shown in FIG. 5) lie beneath the cross-bars of the leads 67bl and 67br, respectively. The bottom left Lamb-wave transducer 60bl consists of a plurality of interlaced fingers which are electrically connected to a pair of transducer leads 64blx and 64bly. The top left and right transducers 60tl and 60tr and the bottom left and right transducers 60bl and 60br have similar shapes. The bottom transducers 60bl and 60br and the bottom transducer leads 64bly, 64blx, 64bry and 64brx, and the leads 56bl, 56br, 67bl and 67br are aluminum and have a thickness of preferably 2000 to 6000 angstroms, and more preferably a thickness of

4000 angstroms, plus or minus ten percent. The bottom transducers 60bl and 60br and leads 56bl, 56br, 67bl, and 67br may be alternatively be made of low-temperature oxide, or any other appropriate conducting material. The top transducers 60tl and 60tr and leads 56tl, 56tr, 67tl, and 67tr may be similarly constructed.

As shown in FIGS. 3 and 4(f), a bottom piezoelectric layer 62b extending between and covering leads 67bl and 67br, fingers of the bottom transducers 60bl and 60br, and portions of leads 56bl and 56br is then deposited and patterned. Similarly, a top piezoelectric layer 62t extending between and covering leads 67tl and 67tr, fingers of the bottom transducers 60tl and 60tr, and portions of leads 56tl and 56tr is then deposited and patterned. The piezoelectric material may be ZnO or any other appropriate material. The thickness of the piezoelectric sections 62t and 62b is preferably between 0.5 and 2.0 μm, more preferably 1 μm, plus or minus ten percent.

The piezoelectric sections 62t and 62b are covered by top and bottom conducting ground planes 66t and 66b, respectively, as shown in FIGS. 3 and 4(f). The ground planes 66t and 66b may be aluminum, or any other appropriate conducting material. The thickness of the conducting ground plane layers 66t and 66b is preferably between 2000 and 6000 angstroms, and more preferably 4000 angstroms, plus or minus ten percent.

Lamb waves, also known as plate-mode waves, have a compressional component and a shear component. Together the components of a traveling Lamb-wave in a slab can transport liquids and gases adjacent the

slab, much the same way ocean waves transport a surfing board. Lamb-wave devices can therefore act as pumps. Material near the surface of the slab has the maximum velocity since there are essentially no boundary layer effects. This is extremely advantageous for the small geometries associated with microdevices. FIG. 6a shows the typical flow velocity of a fluid through a conduit with two planar walls to illustrate the effect of the friction induced by walls on the flow. The velocity profile is parabolic, and at the left and right edges of the conduit the velocity drops to zero due to friction between the fluid and the walls. The friction between the walls and the fluid reduces the efficiency of the pumping. In contrast, FIG. 6b shows the flow velocity of a fluid through the same conduit when the front and back walls are Lamb-wave pumps. The flow velocity is almost constant between the right and left walls. The viscosity of the fluid transmits the momentum of the fluid near the Lamb-wave pumps to the fluid farther from the pumps.

Lamb waves require a propagation medium which is at most several wavelengths thick. The Lamb waves in this invention have frequencies in the approximate range of 1 to 200 MHz, and typical pumping velocities for a Lamb-wave device operated with a 10 volt transducer voltage are 300 μm/sec for water, and 2 cm/sec for air.

The layers 54t, 58t, 62t and 66t which border the top of the chamber cavity 31 will hereinafter be referred to as the ceiling 70t of the chamber 30, and the layers 54b, 58b, 62b and 66b which border the bottom of the chamber cavity 31 will hereinafter be referred to as the floor 70b of the chamber 30. Lamb waves are generated in the ceiling

70t of the chamber 30 by applying an alternating voltage between a transducer lead, for instance the upper left lead 64tly of F 3. 5, and the top ground plane 66t. The transducer electrodes 60tly differentially deform the piezoelectric material 62t to produce a mechanical wave motion in the ceiling 70t. The amplitude of the Lamb waves is increased by applying a second alternating voltage which is 180° out of phase to the transducer lead 64tlx connected to the set of interlaced fingers.

Traveling waves are generated in the Lamb-wave pumps LW_1, LW_2, LW_3, and LW_{DP} in the directions of the arrows of FIG. 2 by application of alternating voltages to the pumps LW_1, LW_2, LW_3, and LW_{DP} at the arrow-tail side. Standing waves are generated in the Lamb-wave detector LW_D by application of in-phase alternating voltages electrodes at both sides of the chamber. By sending Lamb-waves from left to right across the ceiling 70t of the mixing chamber 30 (by application of an alternating voltage between top left transducer 60tl and the top ground plate 66t) and Lamb waves from right to left across the floor 70b of the chamber 30 (by application of an alternating voltage between bottom right transducer 60br and the bottom ground plate 66b), a stirring or circulating action can be produced.

The phenomenon responsible for the operation of the Lamb-wave sensor LW_D in the detection chamber 34 is elastic wave propagation along a medium whose characteristics can be altered by a measurand, such as viscosity or density of the ambient fluid or gas. Where the characteristics of the waves propagating along the medium are dependent upon the characteristics of the propagation medium, the wave characteristics can be monitored or measured

to provide an indication of the measurand value. For example, when the device absorbs vapors or gases from the atmosphere in a film deposited on the surface, the output frequency changes. Tests and analysis indicate that Lamb-wave sensors are at least an order of magnitude more sensitive than other types of acoustical vapor sensors operating at the same wavelength. The type of DNA in an ambient fluid can be determined by measuring the viscosity as a function of temperature, since the denaturing temperatures of different types of DNA are well known.

Since Lamb-wave sensors can operate while in contact with a liquid such as water, their use as biosensors is very significant. For instance, the surface of a Lamb-wave sensor may be coated with single strands of a particular DNA molecule. If the device is immersed in a solution containing that same type of DNA molecule, the molecules in solution will pair with the molecules on the surface, increasing the mass of the membrane and therefore decreasing the frequency of oscillation. Also, the membrane may be made of a porous and permeable material, allowing the coating of a greater surface area and also allowing the liquid to be flowed through the membrane, in order to speed up the DNA attachment. Intercalating dyes, such as ethidium bromide, may be used to augment viscosity changes which occur during a reaction, thereby increasing the sensitivity of the sensor. Other biological interactions may also be sensed.

Figure 7 shows gel electrophoresis results verifying the amplification of a specific HIV nucleic acid sequence performed by the microfabricated reaction instrument of the present invention. The

two outside bands C_1 and C_2 represent calibrating size standards, and the three bands labelled as D_1 represent the DNA amplified in the microreactor. The two bands E_1 to the left of center are results obtained with a commercial PCR thermocycler instrument from the same reaction mixture as that in the microreactor. Since bands E_1 and D_1 are at the same height it is indicated that the microreactor has produced the correct target. The thermal cycles of the commercial instrument were 4 minutes long. Those of the microreactor of the present invention were 1 minute in length.

In summary, an apparatus and method for *in situ* chemical reactions in a microfabricated instrument has been described. It has been shown that the instrument facilitates extremely rapid thermal cycling and high-precision temperature control of microliter to nanoliter volumes. The apparatus and method are well suited for DNA-based reactions, such as PCR. It has also been shown that such integrated devices minimize the possibility for contamination of the reactions and may be operated at a distance by electromagnetic fields.

The present invention has been described in terms of a preferred embodiment. However, the invention is not limited to the embodiment depicted and described. Many variations within the scope of the present invention are possible. For instance, the instrument may consist of a series of chambers at different temperatures, and the temperature programming of the reagents may be accomplished by transport of the reagents through the series of chambers. The instrument may have an input channel and an output channel, and may be adapted to provide a continual flow-through synthesis. Thermal cycling

may be accomplished by repeated transfer of reagents between two or more chambers which are heated to different temperatures.

In another alternate embodiment the monitoring of the temperature of a chamber is accomplished at a distance by connecting an LC circuit to a probe across a polycrystalline heating layer and measuring the Q-factor of the circuit. The Q-factor is measured by exciting the circuit with an incident electromagnetic field at the resonant frequency of the circuit and monitoring the time decay of the resonance, or by measuring the bandwidth of the circuit by applying a frequency modulated incident EM field and measuring the circuit's frequency response. Since the measured Q-factor is inversely proportional to the resistance and the resistance increases linearly with temperature, the temperature may be determined by the measured Q-factor.

Other possible variations within the spirit of the invention include: the dimensions of components of the instrument are not limited to those disclosed; more or fewer reactants may be used and the reactants may be organic, inorganic, or a combination of organic and inorganic; the detection means may be located in the same chamber as the mixing means; components of the device may be made of semiconducting materials other than silicon, or of quartz, glass, polymers or other materials; the microfabricated instrument may be formed by the bonding of two wafers; the instrument may be provided with optical windows for optical monitoring of the reaction; the instrument may be controlled by direct electrical coupling of control circuitry to the leads of the pumps, heater and sensor; a

silicone-rubber window may form a penetrable wall of any chamber or channel; the instrument may be fabricated from a silicon-based material; the instrument may be powered by an integrated microfabricated battery; any Lamb-wave transducer may be activated by capacitive, inductive, electromagnetic or optical means; or electrokinetic pumps, or any other appropriate type of pumping means, may be substituted for the Lamb-wave pumps.

Accordingly, the scope of the invention is defined by the appended claims.

WHAT IS CLAIMED IS:

1. An instrument for controlling a chemical reaction of reagents comprising: integrated microfabricated elements, said elements including a first reagent chamber for containment of a first one of said reagents, and a means for manipulation of a parameter of said reaction.

2. The instrument of claim 1 wherein said microfabricated elements include a control means for controlling said means for manipulation, and a power source for powering said control means.

3. The instrument of claim 2 wherein said power source comprises a battery.

4. The instrument of claim 1 further including a control means for controlling said means for manipulation, said control means being coupled to said means for manipulation by optical radiation.

5. The instrument of claim 1 further including a control means for controlling said means for manipulation, said control means being coupled to said means for manipulation by electromagnetic radiation.

6. The instrument of claim 1 further including a control means for controlling said means for manipulation, said control means being coupled to said means for manipulation by an electromagnetic field.

7. The instrument of claim 6 wherein said electromagnetic field is substantially comprised of an electric field.

8. The instrument of claim 6 wherein said electromagnetic field is substantially comprised of a magnetic field.

9. The instrument of claim 1 wherein said instrument has an exterior surface, further including an optical window between said first reagent chamber and said exterior surface, whereby exterior light may be directed to contents of said first reagent chamber through said window.

10. The instrument of claim 1 wherein one of said elements comprises a Lamb-wave transducer at a boundary of said first chamber.

11. The instrument of claim 1 wherein said means for manipulation comprises an ultrasound transducer.

12. The instrument of claim 1 wherein said means for manipulation comprises an electromagnetic radiation transducer.

13. The instrument of claim 1 wherein said means for manipulation comprises a means for heating said first chamber.

14. The instrument of claim 13 wherein said means for heating comprises a resistive element near said first chamber.

15. The instrument of claim 14 wherein said resistive element is substantially thermally isolated.

16. The instrument of claim 14 wherein said resistive element is substantially thermally isolated by an envelope of a low-stress material.

17. The instrument of claim 16 wherein a portion of said envelope forms a portion of a wall of said chamber.

18. The instrument of claim 17 wherein said low-stress material is low-stress silicon nitride.

19. The instrument of claim 1 further including a boundary layer forming a wall of said chamber, said boundary layer being comprised of a low-stress material.

20. The instrument of claim 19 wherein said low-stress material is low-stress silicon nitride.

21. The instrument of claim 14 further comprising a resonant circuit electrically connected to said resistive element, a means for exciting said resonant circuit at a distance, and a means for monitoring the energy stored in said resonant circuit at a distance, whereby the Q-factor of said resonant circuit may be monitored at a distance, thereby determining the resistance of said resistive element and the temperature of said resistive element.

22. The instrument of claim 13 wherein said means for heating produces a predetermined sequence of temperatures in said first chamber.

23. The instrument of claim 13 wherein said means for heating produces a number of thermal cycles in said first chamber.

24. The instrument of claim 23 wherein said number is greater than ten.

25. The instrument of claim 23 wherein said number is greater than twenty five.

26. The instrument of claim 23 wherein one of said elements comprises a second reagent chamber, and one of said elements comprises a means for pumping said first reagent between said first chamber and said second chamber.

27. The instrument of claim 26 wherein said means for pumping comprises a Lamb-wave transducer.

28. The instrument of claim 26 wherein said means for pumping comprises a differential temperature pump.

29. The instrument of claim 1 wherein one of said elements comprises a second reagent chamber, and one of said elements comprises a means for pumping said first reagent between said first chamber and said second chamber.

30. The instrument of claim 29 wherein said means for pumping comprises a Lamb-wave transducer.

31. The instrument of claim 1 wherein one of said elements comprises a means for determining the progress of said reaction.

32. The instrument of claim 31 wherein said means for determining comprises a sensor for monitoring the viscosity of one of said reagents.

33. The instrument of claim 32 wherein one of said reagents comprises an intercalating molecule which augments a viscosity change associated with said reaction.

34. The instrument of claim 33 wherein said intercalating molecule intercalates into a deoxyribonucleic acid.

35. The instrument of claim 33 wherein said sensor comprises a flexural wave sensor.

36. The instrument of claim 32 wherein said sensor comprises a flexural wave sensor.

37. The instrument of claim 33 wherein said reagents include a deoxyribonucleic acid.

38. The instrument of claim 33 wherein said reagents include a ribonucleic acid.

39. The instrument of claim 33 wherein said reagents include a polymer.

40. The instrument of claim 33 wherein said reagents include a large molecule.

41. The instrument of claim 33 wherein said reagents include a protein.

42. The instrument of claim 27 wherein one of said chambers has a capacity of less than a milliliter.

43. The instrument of claim 27 wherein one of said chambers has a capacity of less than fifty microliters.

44. The instrument of claim 27 wherein one of said chambers has a capacity of less than a microliter.

45. The instrument of claim 26 wherein one of said chambers has a capacity of less than a picoliter.

46. The instrument of claim 1 wherein one of said elements comprises a means for stirring the contents of said first chamber.

47. The instrument of claim 46 wherein one of said elements comprises a Lamb-wave transducer at a boundary of said chamber, thereby preventing boundary layer effects from reducing the effectiveness of the stirring.

48. The instrument of claim 1 wherein one of said elements comprises an elastomeric window interposed between a boundary of said first reagent chamber and the exterior of said instrument, whereby a second reagent may be inserted into said first chamber by puncturing said window with a hollow needle.

49. The instrument of claim 1 further including a hypodermic needle means for extracting a body fluid, said needle means having a fluid conduit terminating at said first chamber.

50. The instrument of claim 1 wherein said microfabricated elements are formed on a semiconducting substrate.

51. The instrument of claim 50 wherein said semiconducting substrate is composed of a silicon-based material.

52. The instrument of claim 1 wherein said microfabricated elements are formed on a substrate composed of a polymer.

53. The instrument of claim 1 wherein said microfabricated elements are formed on a substrate composed of a glass.

54. The instrument of claim 1 wherein one of said reagents is an organic compound.

55. The instrument of claims 1 wherein one of said reagents is an inorganic compound.

56. The instrument of claim 1 wherein one of said reagents is a biochemical compound.

57. The instrument of claim 1 wherein one of said reagents is a nucleic acid.

58. The instrument of claim 1 wherein one of said reagents is a protein.

59. The instrument of claim 1 wherein said chemical reaction is a chain reaction.

60. The instrument of claim 59 wherein said chain reaction is a polymerase chain reaction.

61. The instrument of claim 59 wherein said chain reaction is a ligase chain reaction.

62. The instrument of claim 1 wherein said chemical reaction is comprised of a plurality of substantially similar subreactions.

63. The instrument of claim 62 wherein said chemical reaction produces a quantity of a target product.

64. The instrument of claim 63 wherein said quantity of said target product increases exponentially with each said subreaction.

65. The instrument of claim 63 wherein said target product increases linearly with each said subreaction.

66. The instrument of claim 56 wherein said chemical reaction is a polymerase chain reaction.

67. An instrument for controlling a chemical reaction, said chemical reaction producing a product from a set of reagents, said instrument comprising: integrated microfabricated elements, said elements including an array of chambers for containment of reagents of said reaction, a plurality of channels interconnecting said chambers, and a means for transferring said reagents between said chambers by way of said channels.

68. The instrument of claim 67 wherein said elements further include a first heating means for maintaining a first one of said chambers at a first temperature and a second heating means for maintaining a second one of said chambers at a second temperature.

69. The instrument of claim 67 wherein said instrument has an exterior surface and further including an optical window between a first selected chamber and said exterior surface.

70. The instrument of claim 68 wherein said means for transferring includes means for repeatedly transferring said reagents located in said first chamber to said second chamber and repeatedly transferring said reagents located in said second chamber to said first chamber.

71. The instrument of claim 67 further including a means for manipulation of a parameter of said reaction.

72. The instrument of claim 67 wherein said chambers in said array are interconnected substantially in series by said channels.

73. The instrument of claim 72 further comprising an input channel and an output channel, a subset of said reagents being continually introduced to said instrument through said input channel, and said product being continually extruded from said instrument through said output channel.

74. The instrument of claim 73 further including a plurality of heaters, said heaters being located proximate said chambers and maintaining said chambers at a plurality of temperatures, whereby transport of said reagents through said chambers subjects said reagents to said plurality of temperatures.

75. The instrument of claim 67 wherein said means for transferring comprises an active pumping means.

76. The instrument of claim 75 wherein said active pumping means comprises a Lamb-wave pump.

77. The instrument of claim 67 wherein said means for transferring comprises a passive pumping means.

78. The instrument of claim 77 wherein said passive pumping means comprises a heating means proximate a chamber selected from said array of chambers for heating contents of said selected chamber, whereby said contents may be forced from said selected chamber by heating said contents with said heating

means and thereby increasing the volume of said contents.

79. The instrument of claim 67 wherein said array of chambers is comprised of a plurality of substantially similar groups of chambers.

80. The instrument of claim 79 wherein chambers within a first group are fluidicly interconnected, and chambers within said first group are substantially isolated from chambers within a second group.

81. A process for manufacturing on a wafer an instrument for controlling a chemical reaction, comprising the steps of etching said wafer to form a reaction chamber, and depositing a resistive element on said wafer adjacent a boundary of said reaction chamber.

82. The process of claim 81 further comprising the steps of etching a reagent chamber and etching a passage from said reagent chamber to said reaction chamber.

83. The process of claim 81 further comprising the step of depositing a Lamb-wave transducer on said wafer.

84. The process of claim 83 wherein said Lamb-wave transducer is located near a boundary of said reaction chamber.

85. The process of claim 81 further comprising the step of depositing a window of an elastomeric material interposed between a boundary of one of said chambers and the exterior of said wafer.

86. The process of claim 81 wherein said wafer comprises a semiconductor.

87. The process of claim 86 wherein said semiconductor comprises a silicon-based material.

88. A microfabricated mixing chamber comprising at least one Lamb-wave transducer for stirring contents of said chamber, thereby preventing boundary layer effects from reducing the effectiveness of the stirring.

89. A microfabricated chamber comprising at least one Lamb-wave transducer for viscosity measurements of contents of said chamber, thereby preventing boundary layer effects from reducing the effectiveness of the stirring.

90. An instrument for controlling a chemical reaction of reagents comprising: integrated microfabricated elements, said elements including

a reagent chamber for containment of a first one of said reagents, said chamber having a first wall and a second wall;

a first Lamb-wave transducer located near said first wall; and

a second Lamb-wave transducer located near said second wall.

91. The instrument of claim 90 wherein said first and second walls are located at opposite sides of said chamber.

92. The instrument of claim 91 wherein said first Lamb-wave transducer has two sets of interlaced transduction leads, and said second Lamb-wave

transducer has two sets of interlaced transduction leads.

ABSTRACT

An integrated microfabricated instrument for manipulation, reaction and detection of microliter to picoliter samples. The instrument is suited for biochemical reactions, particularly DNA-based reactions such as the polymerase chain reaction, that require thermal cycling since the inherently small size of the instrument facilitates rapid cycle times. The integrated nature of the instrument provides accurate, contamination-free processing. The instrument may include reagent reservoirs, agitators and mixers, heaters, pumps, and optical or electromechanical sensors. Ultrasonic Lamb-wave devices may be used as sensors, pumps and agitators.

Fig. 1

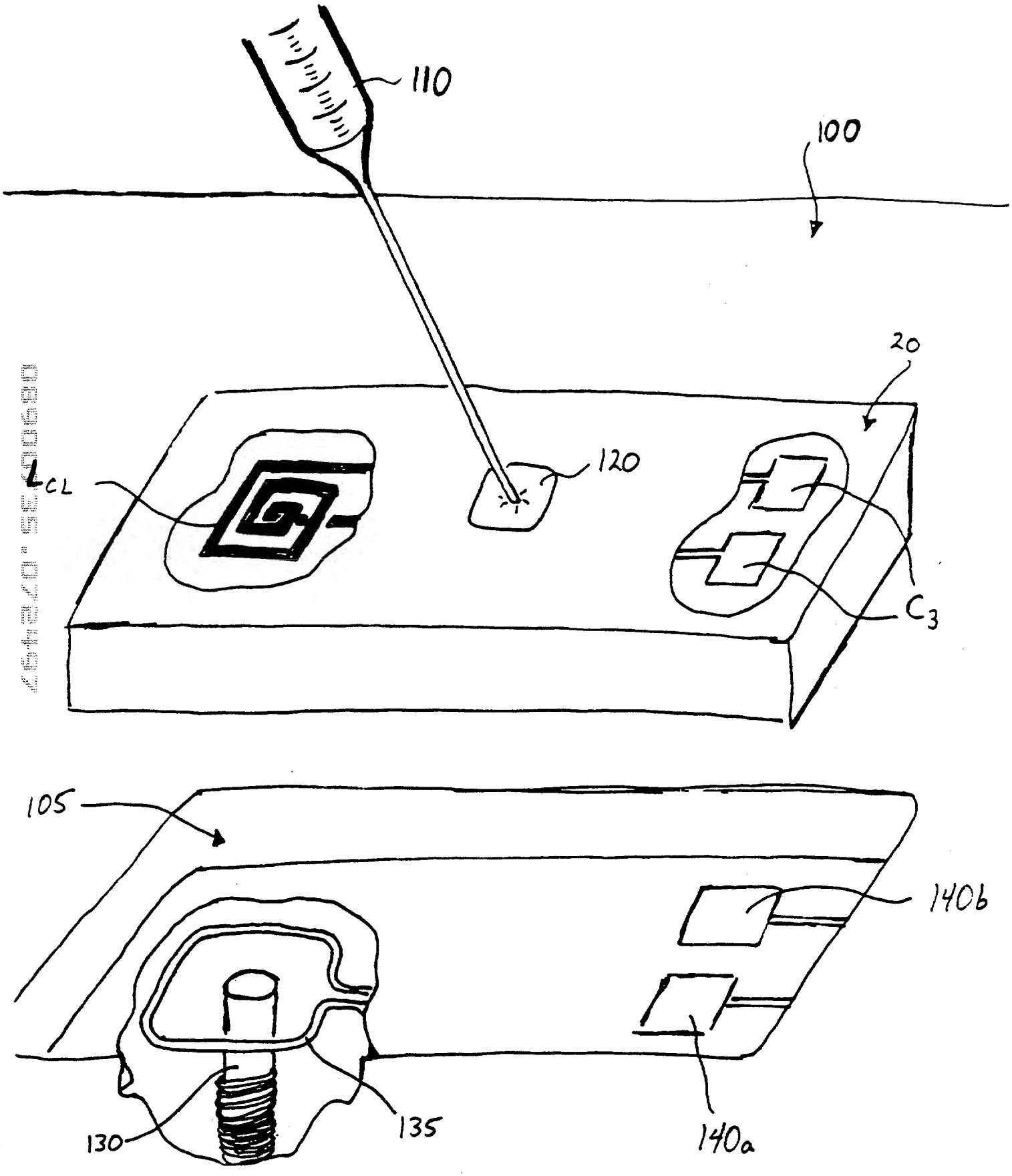
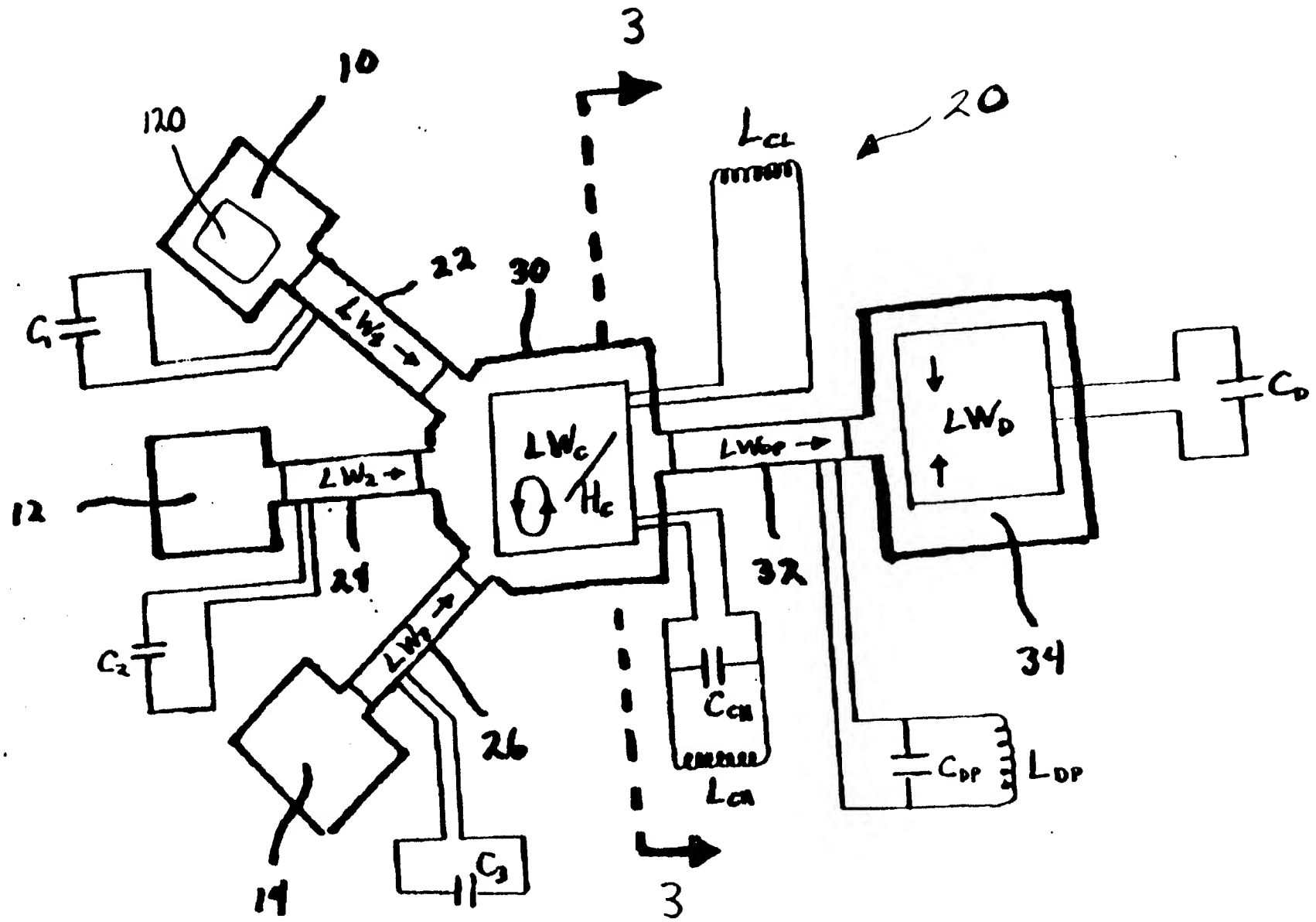


FIG. 2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22
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LW_1
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H_C
LW_{CP}
LW_D
C_D
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LW_2
21
32
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19
11
C_3
3

FIG. 3

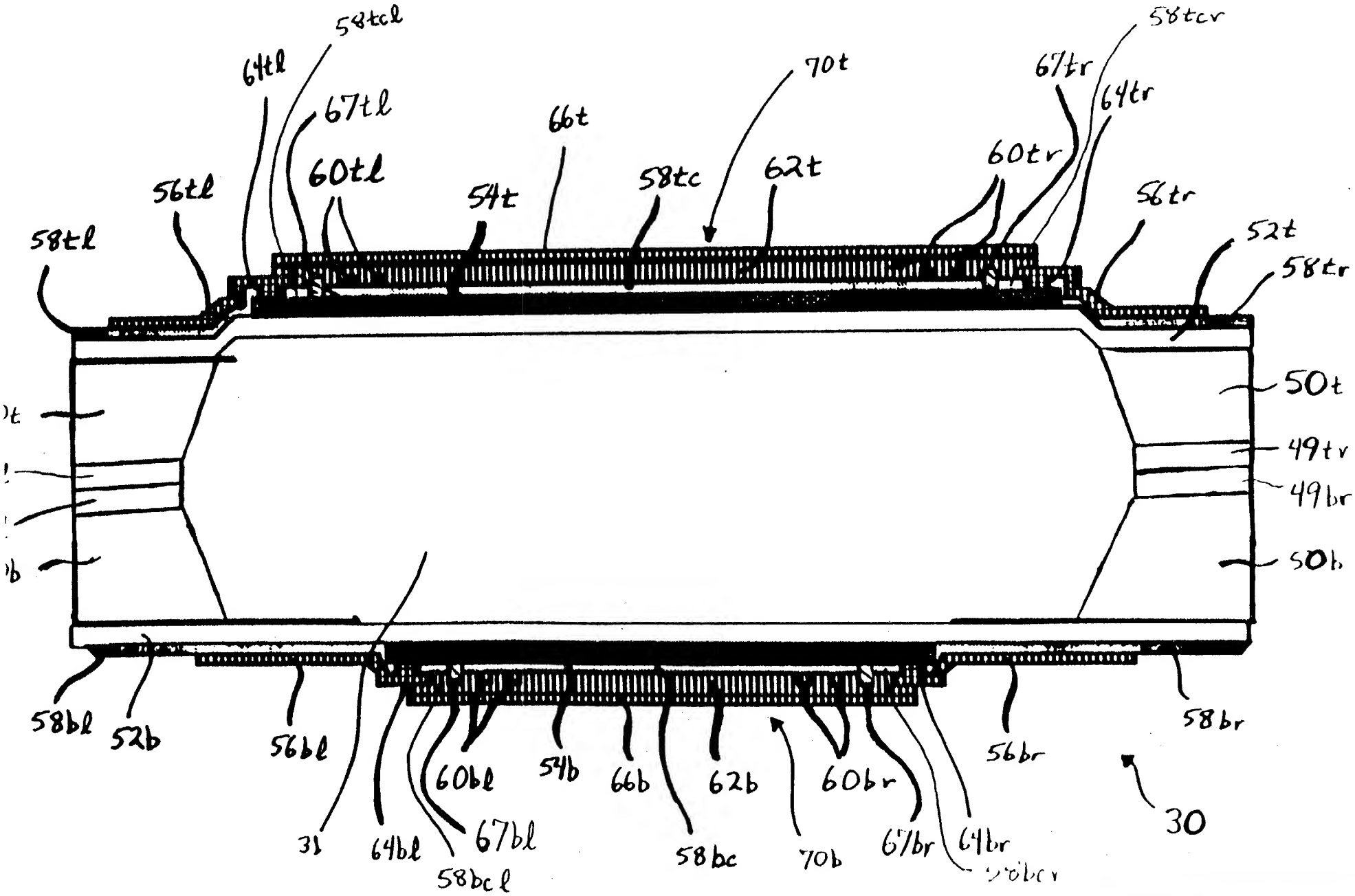
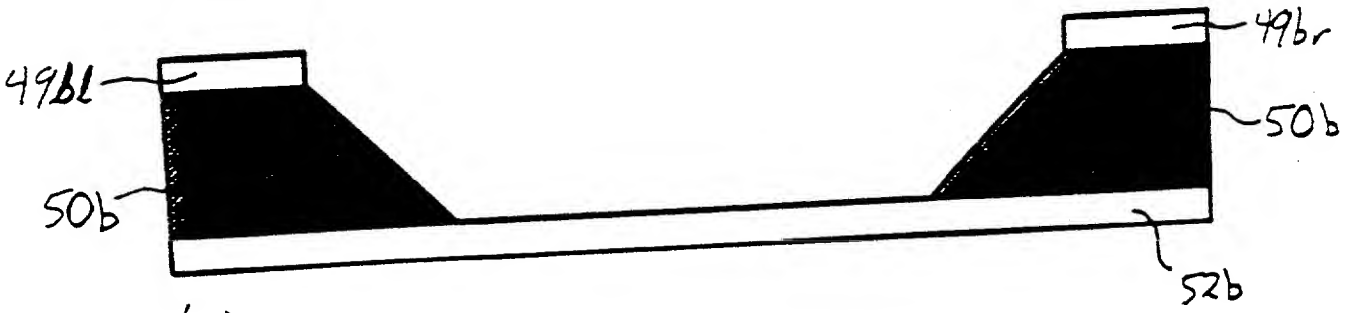
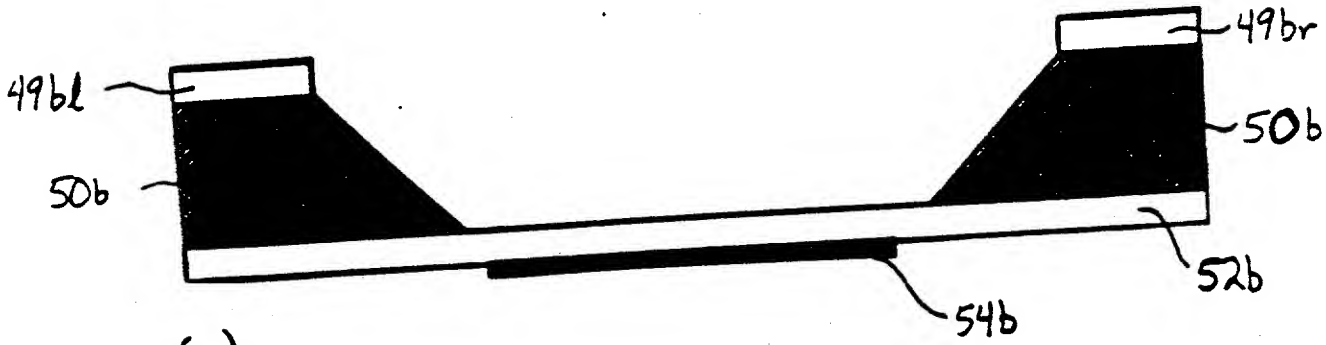


FIG. 4

(a)

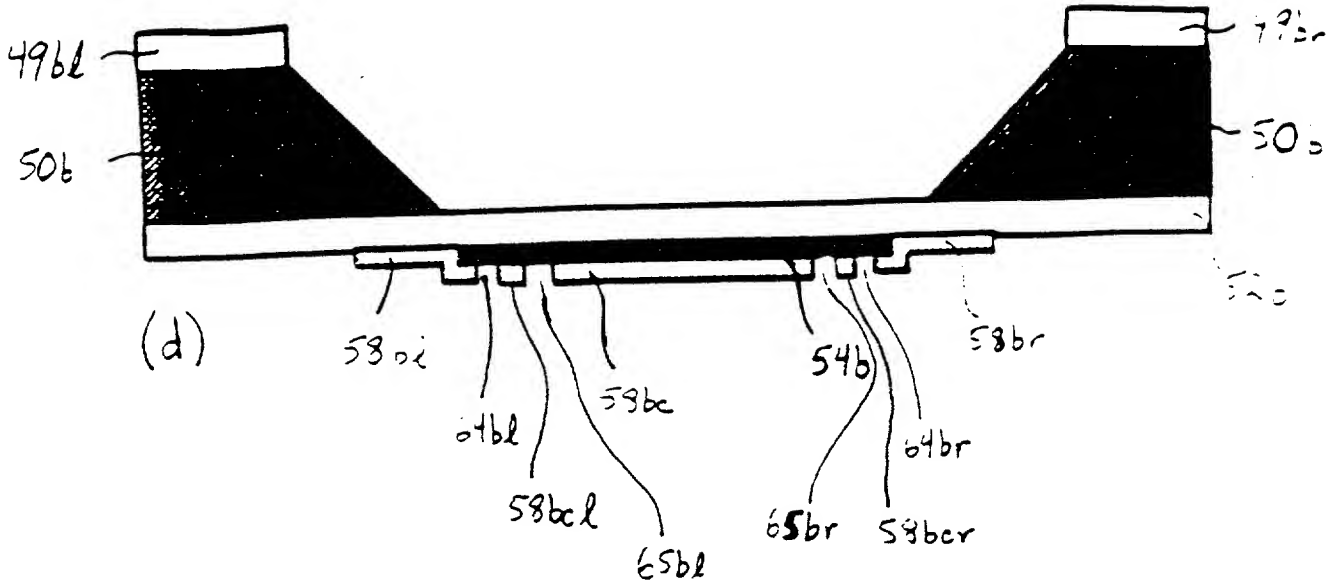


(b)



(c)

FIG. 4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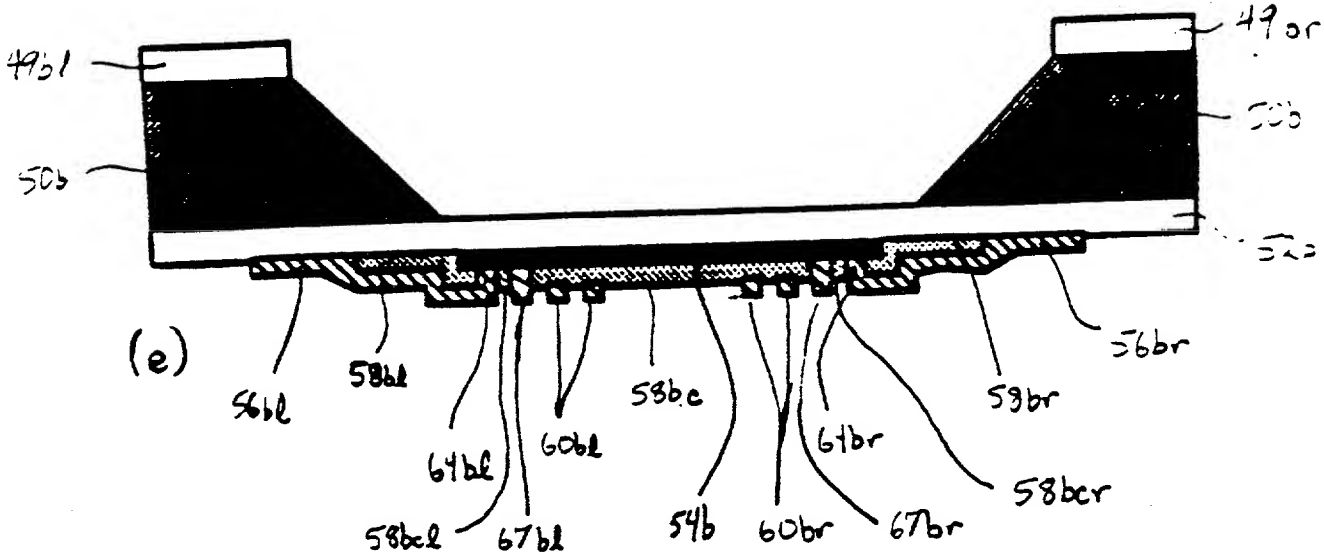
64br
58br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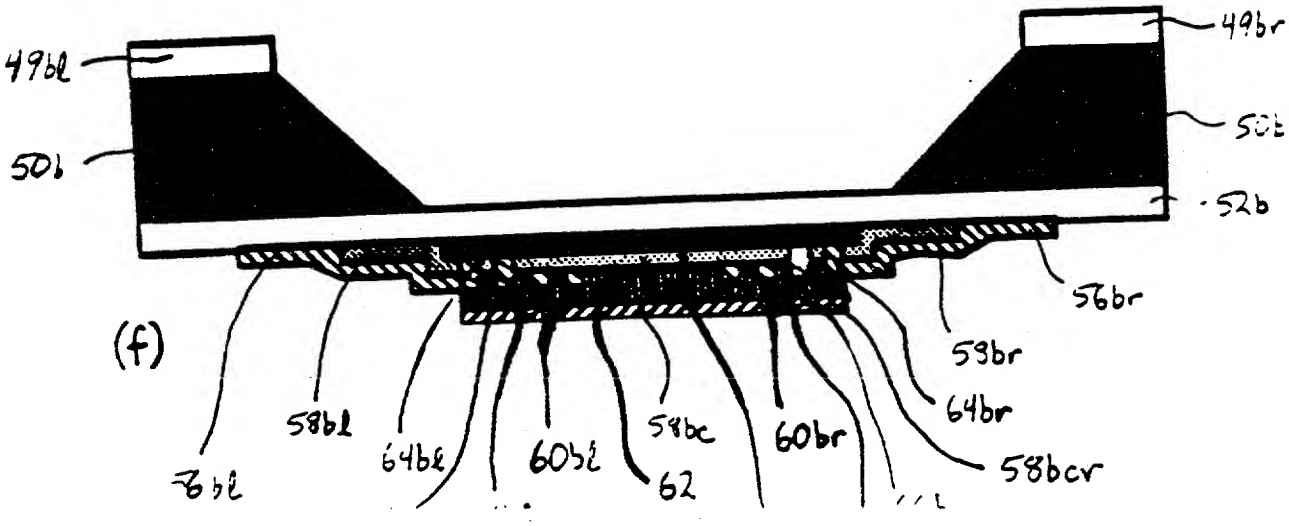
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50b
49br
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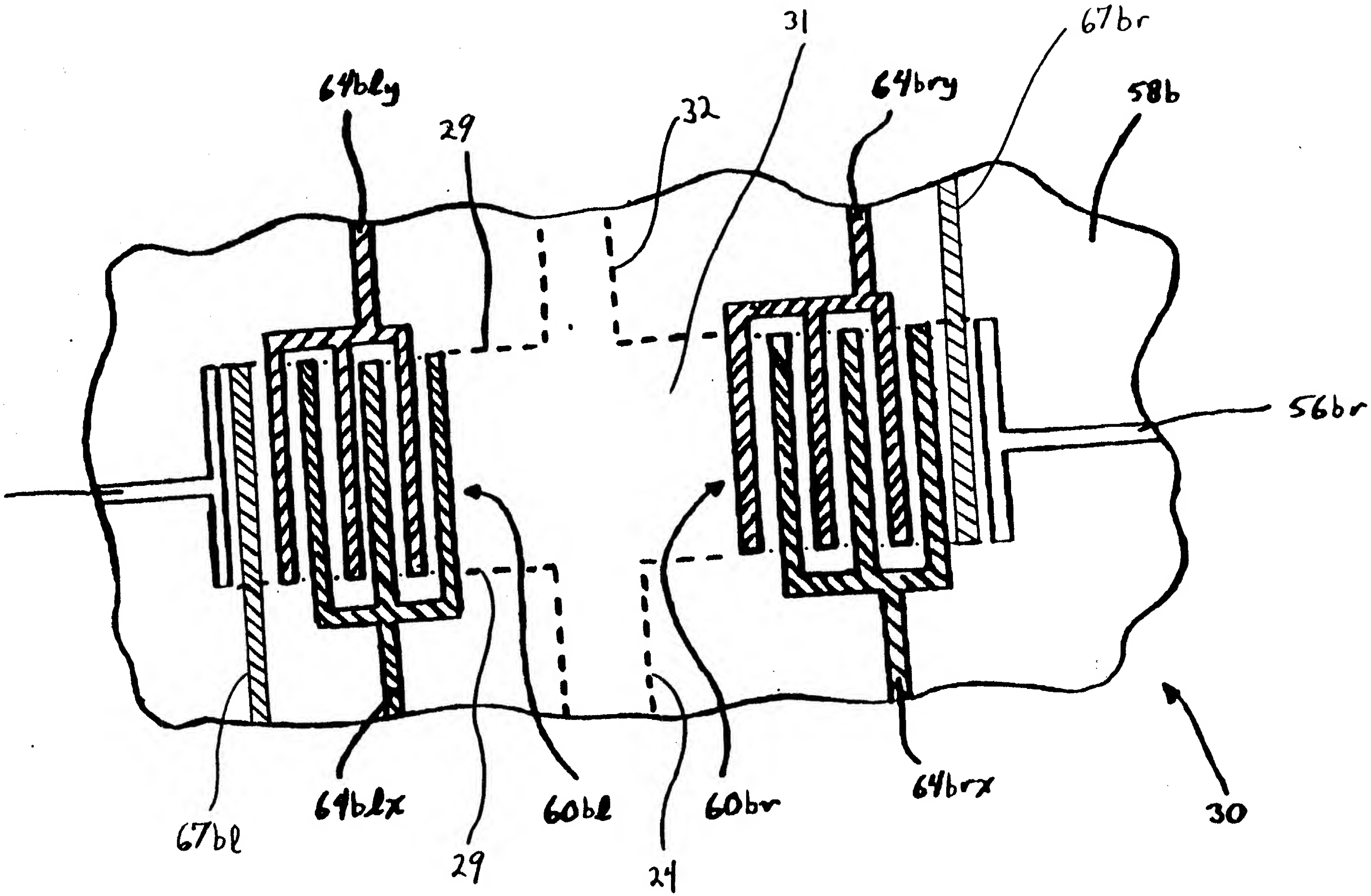
60bl
62
58bc
60br
59br
64br
58bcr
56br

FIG. 5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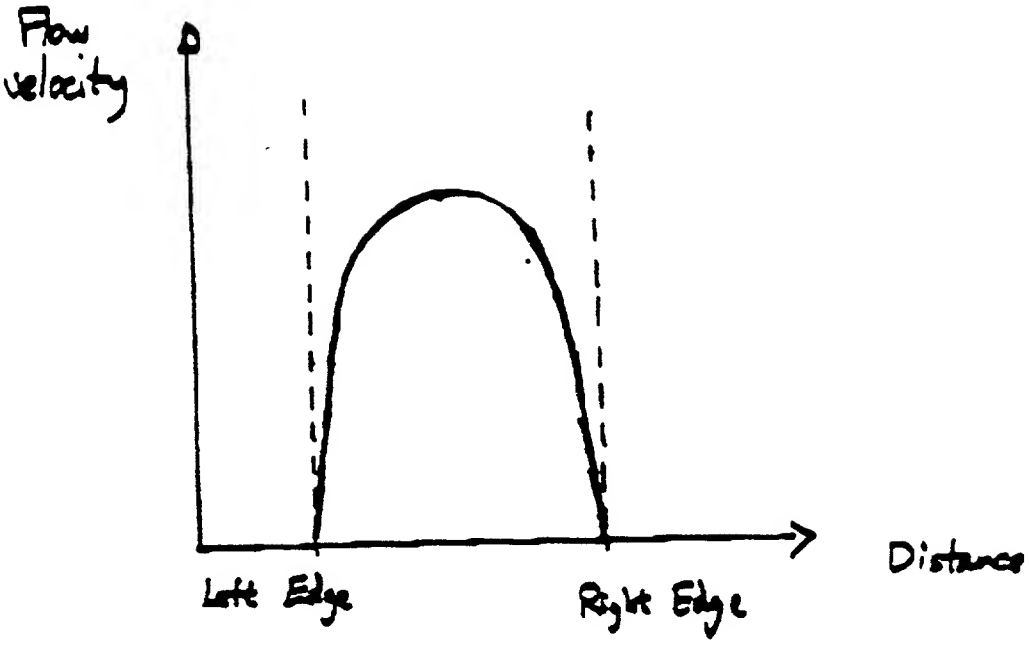
Flow velocity
Distance
Left Edge
Right Edge

Fig. 6a

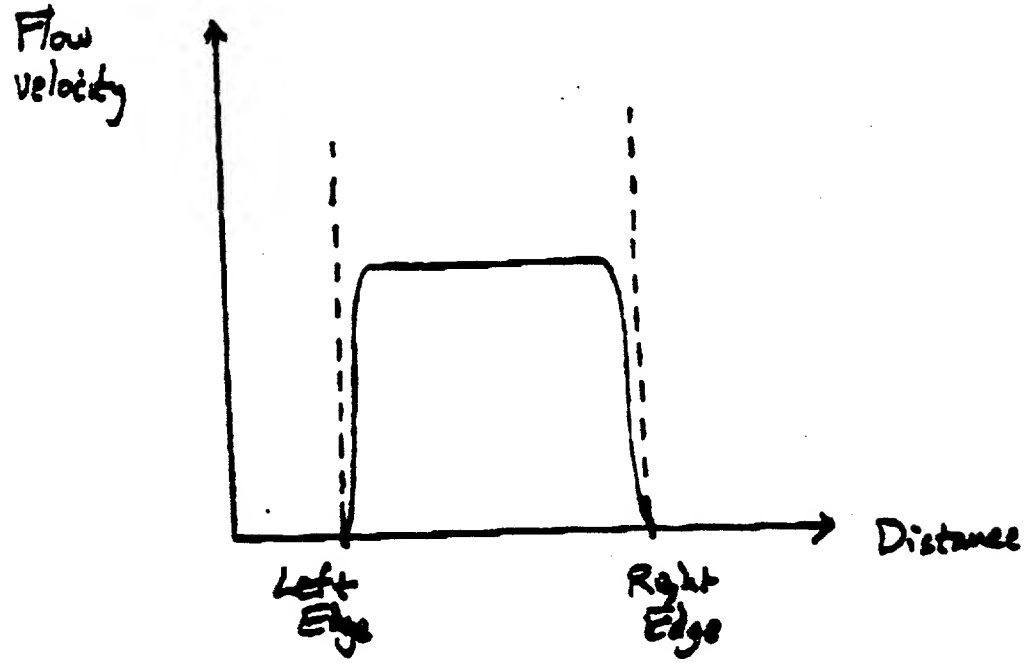
Flow velocity
Distance
Left Edge
Right Edge

Fig 6b

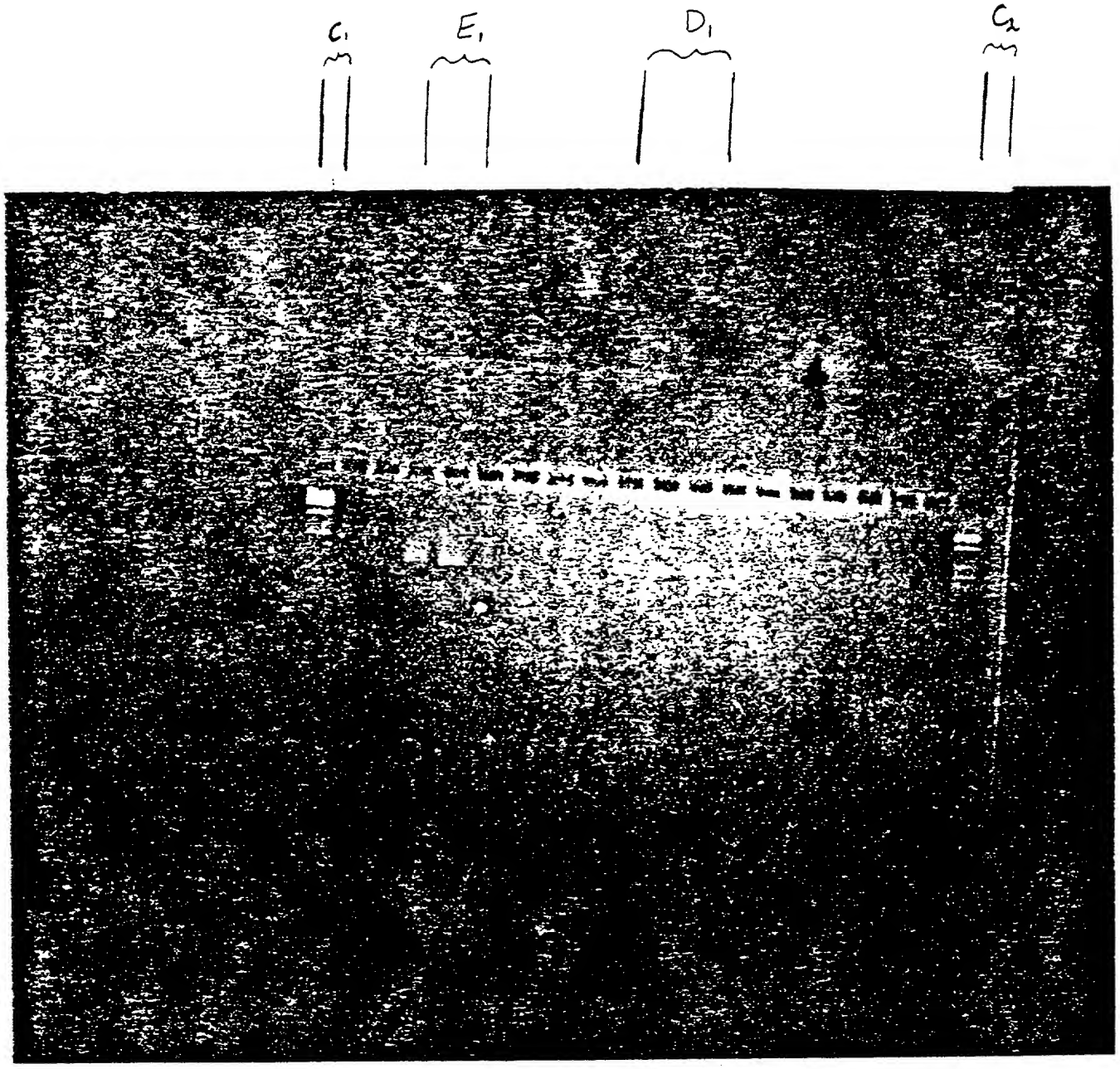

Fig. 7

POWER OF ATTORNEY BY ASSIGNEE
AND EXCLUSION OF INVENTOR UNDER 37 C.F.R. 1.32
(Not Accompanying Application)

To the Commissioner of Patents and Trademarks:

The undersigned assignee of the entire interest in application for letters patent entitled: MICROFABRICATED REACTOR and having the named inventors: M. ALLEN NORTHRUP and RICHARD M. WHITE, Serial No. 07/938,106 filed on the 31st day of August, 1992, hereby appoints the following attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith; said appointment to be to the exclusion of the inventor(s) and his (their) attorney(s) in accordance with the provisions of 37 C.F.R. 1.32:

Stephen E. Baldwin, Reg. No. 27,769, William J. Egan, III, Reg. No. 28,411, Keiichi Nishimura, Reg. No. 29,093, Reginald J. Suyat, Reg. No. 28,172;

provided that if any one of said attorneys ceases being affiliated with the law firm of HELLER, EHRMAN, WHITE & McAULIFFE as partner, employee or of counsel, such attorney's appointment as attorney and all powers derived therefrom shall terminate on the date such attorney ceases being so affiliated.

Direct all telephone calls to WILLIAM J. EGAN, III at (415) 772-6845.

Address all correspondence to:

William J. Egan, III, Esq.
HELLER, EHRMAN, WHITE & McAULIFFE
333 Bush Street
San Francisco, California 94104-2878

Assignee: THE REGENTS OF THE UNIVERSITY OF CALIFORNIA

By: William A. Hoskins
(typed name)

Date: Oct 1, 1992

Signature: [signature]

Title: Director

Address: Office of Technology Licensing
2150 Shattuck Avenue, Suite 510
Berkeley, California 94704

HELLER, EHRMAN, WHITE & McAULIFFE
Attorney Docket No. 18043-0021

DECLARATION FOR PATENT APPLICATION

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled MICROFABRICATED REACTOR, the specification of which

(check one)

☐ is attached hereto.

☒ was filed on August 31, 1992 as Application Serial No. 07/938,106 and was amended on ________________. (if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed	
________	________	________	☐	☐
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No
________	________	________	☐	☐
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No
________	________	________	☐	☐
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in §1.56, which became available between the filing date of the prior application and the national or PCT international filing date of this application:

________	________	________
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
________	________	________
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

Direct all telephone calls to WILLIAM J. EGAN, III at (415) 772-6845.

Address all correspondence to:

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333 Bush Street
San Francisco, California 94104

Our File No. 18043-0021

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Title 18, United States Code, §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor: M. ALLEN NORTHRUP

Inventor's signature: M. Allen Northrup

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Citizenship: U.S.A.

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Inventor's signature: R. M. White

Date: October 1, 1992

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